

**WHAT IS CLAIMED IS:**

1. A fluorescence spectrophotometer system comprising:
  - a light source;
  - a first double monochromator comprising two or more gratings and operative to separate light from the light source into a plurality of wavelengths and to output selected wavelengths as excitation light;
  - a light transfer module comprising:
    - a first reflection surface operative to direct substantially all of the excitation light directly onto a sample; and
    - a second reflection surface operative to direct light that is emitted from the sample as fluorescent or luminescent light;
  - a second double monochromator comprising two or more gratings and operative to separate the fluorescent or luminescent light directed by the light transfer module into a plurality of wavelengths and to output selected wavelengths of the fluorescent or luminescent light as emission light; and
  - a photodetector and analyzer, operative to receive the emission light output by the second double monochromator, to detect the selected wavelengths of the emission light, and to output an indication of the selected wavelengths.
2. The system of claim 1 wherein at least one of the first double monochromator or the second double monochromator comprises:
  - an entrance aperture for accepting light;
  - a first optical grating positioned to disperse at least part of the light accepted through the entrance aperture;
  - a first selection aperture positioned to intercept part of the light dispersed by the first optical grating and operative to pass selected wavelengths of the dispersed light;
  - a second optical grating positioned to disperse at least part of the light passed through the first selection aperture; and
  - a second selection aperture positioned to intercept part of the light dispersed by the second optical grating and operative to pass selected wavelengths of the dispersed light.
3. The system of claim 2 wherein the first optical grating and the second optical grating are concave.
4. The system of claim 3 wherein the first optical grating and the second optical grating are holographic concave gratings.
5. The system of claim 2 wherein each of the first optical grating and the second optical grating is operative to pivot about a respective axis of rotation

- 35

an optical filter operative to restrict the excitation light to plane polarized excitation light; and  
an optical filter holder selectively operative to insert the optical filter into the path of the excitation light.

18. The system of claim 17 wherein the optical filter and the optical filter holder are incorporated into the first double monochromator.

19. The system of claim 17 wherein the optical filter and the optical filter holder are incorporated into the light transfer module.

20. The system of claim 17 further comprising:

a first polarizing filter operative to transmit emission light in a plane which is parallel to the plane of the polarized excitation light;

a second polarizing filter operative to transmit emission light in any plane which is not parallel to the plane of the polarized excitation light; and

a polarizing filter holder selectively operative to insert one of the first polarizing filter or the second polarizing filter into the path of the emission light.

21. The system of claim 20 wherein the first polarizing filter, the second polarizing filter, and the polarizing filter holder are incorporated into the second double monochromator.

22. The system of claim 20 wherein the first polarizing filter, the second polarizing filter, and the polarizing filter holder are incorporated into the light transfer module.

23. A double monochromator comprising:

an entrance aperture for accepting input light;

a first optical grating positioned to disperse at least part of the light accepted through the entrance aperture;

a first selection aperture positioned to intercept part of the light dispersed by the first optical grating and operative to pass a selected range of wavelengths of the dispersed light;

a second optical grating positioned to disperse at least part of the light passed through the first selection aperture; and

a second selection aperture positioned to intercept part of the light dispersed by the second optical grating and operative to pass a selected range of wavelengths of the dispersed light as output light.

24. The double monochromator of claim 23 wherein the first optical grating and the second optical grating are concave.

25. The double monochromator of claim 24 wherein the first optical grating and the second optical grating are holographic concave gratings.
26. The double monochromator of claim 23 wherein each of the first optical grating and the second optical grating is operative to pivot about a respective axis of rotation allowing selection of a range of wavelengths of light to be passed through the first selection aperture and the second selection aperture, respectively, as a function of rotation angle.
27. The double monochromator of claim 26 further comprising means for pivoting the first optical grating about its respective axis of rotation and pivoting the second optical grating about its respective axis of rotation synchronously.
28. The double monochromator of claim 27 wherein the means for pivoting comprises a band drive mechanism operatively coupled to each of the first optical grating and the second optical grating.
29. The double monochromator of claim 23 further comprising:
  - an optical filter operative to restrict the output light to a selected polarized plane; and
  - an optical filter holder selectively operative to insert the optical filter into the path of the output light.
30. The double monochromator of claim 23 wherein the input light is polarized; the double monochromator further comprising:
  - a first polarizing filter operative to transmit light in a plane which is parallel to the plane of the polarized input light;
  - a second polarizing filter operative to transmit light in any plane which is not parallel to the plane of the polarized input light; and
  - a polarizing filter holder selectively operative to insert one of the first polarizing filter or the second polarizing filter into the path of the input light.
31. A light transfer module comprising:
  - an excitation mirror positioned substantially coaxial with an area to be illuminated and operative to direct incoming light to illuminate the area such that the illuminated area emits fluorescent or luminescent light; and
  - an emission mirror positioned substantially coaxial with the illuminated area and in off-axis alignment with the excitation mirror; wherein the emission mirror is operative to focus and to direct light emitted by the illuminated area as emission light.

32. The light transfer module of claim 31 wherein the emission mirror is a spherical mirror.
33. The light transfer module of claim 31 wherein the excitation mirror and the emission mirror are first-surface mirrors.
- 5 34. The light transfer module of claim 31 further comprising:
  - an optical filter operative to restrict the incoming light to a selected polarized plane; and
  - an optical filter holder selectively operative to insert the optical filter into the path of the incoming light.
- 10 35. The light transfer module of claim 34 further comprising:
  - a first polarizing filter operative to transmit light in a plane which is parallel to the plane of the polarized incoming light;
  - a second polarizing filter operative to transmit light in any plane which is not parallel to the plane of the polarized incoming light; and
  - 15 a polarizing filter holder selectively operative to insert one of the first polarizing filter or the second polarizing filter into the path of the emission light.
- 20 36. The light transfer module of claim 35 wherein the polarizing filter holder is selectively operative to interpose one of the first polarizing filter or the second polarizing filter between the illuminated area and the emission mirror.
- 25 37. A spectrophotometer system comprising:
  - a light source comprising a spherical concave reflector system; the reflector system being telecentric at both ends and fully corrected for third order aberrations;
  - a first multiple-grating monochromator having an entrance aperture; the first multiple-grating monochromator being operative to separate light imaged onto the entrance aperture from the light source into a plurality of wavelengths and to output selected wavelengths as excitation light;
  - 30 a light transfer module comprising:
    - a first reflection surface operative to direct substantially all of the excitation light directly onto a sample; and
    - a second reflection surface; the second reflection surface being a compound parabolic reflective surface and operative to collect and to direct light emitted from the sample as fluorescent or luminescent
    - 35 light;
  - a second multiple-grating monochromator operative to separate the fluorescent or luminescent light into a plurality of wavelengths and to output

selected wavelengths of the fluorescent or luminescent light as emission light;  
and

a photodetector and analyzer, operative to receive the emission light output  
by the second multiple-grating monochromator, to detect the selected  
wavelengths of the emission light, and to output an indication of the selected  
wavelengths.

38. The system of claim 37 wherein the reflector system comprises:  
a plurality of apertures; each of the plurality of apertures being  
operative to alter the cone angle of the light imaged on the entrance  
aperture of the first multiple-grating monochromator; and  
means for selectively inserting one of the plurality of apertures into  
the path of light imaged on the entrance aperture.
39. The system of claim 37 wherein the reflector system comprises an Offner 1:1  
afocal relay.
40. The system of claim 37 wherein each of the first multiple-grating  
monochromator and the second multiple-grating monochromator comprises a  
first concave optical grating and a second concave optical grating.
41. The system of claim 40 wherein the first optical grating and the second  
optical grating are holographic concave gratings.
42. The system of claim 40 wherein each of the first concave optical grating and  
the second concave optical grating is operative to pivot about a respective  
axis of rotation.
43. The system of claim 37 wherein the first reflection surface of the light  
transfer module is an excitation mirror positioned substantially coaxial with a  
well containing the sample and wherein the compound parabolic reflective  
surface is positioned substantially coaxial with the well and is in reflective  
alignment with an emission mirror operative to output light emitted from the  
sample to the second multiple-grating monochromator.
44. The system of claim 43 wherein the first reflection surface of the light  
transfer module is a parabolic first-surface excitation mirror.
45. The system of claim 44 wherein the emission mirror is a first-surface mirror.
46. The system of claim 43 wherein the excitation mirror is positioned to direct  
excitation light into the opening of a selected well in a microwell plate  
containing as many as 5000 discrete microwells.
47. The system of claim 43 wherein the excitation light is plane polarized and  
wherein the light transfer module further comprises:

a first polarizing filter operative to transmit light in a plane which is parallel to the plane of the polarized excitation light;  
a second polarizing filter operative to transmit light in any plane which is not parallel to the plane of the polarized excitation light; and  
a polarizing filter holder selectively operative to interpose one of the first polarizing filter or the second polarizing filter between the compound parabolic reflective surface and the emission mirror.

48. The system of claim 37 wherein the light transfer module, the second multiple-grating monochromator, and the photodetector and analyzer are operative to analyze more than one fluorescent compound in the sample.
49. The system of claim 46 further comprising means for translating the microwell plate relative to the light transfer module allowing analysis of samples from selected ones of a plurality of wells in the microwell plate.
50. A light transfer module comprising:
  - an entrance aperture for admitting excitation light;
  - an excitation mirror positioned substantially coaxial with an area to be illuminated and operative to direct excitation light from the entrance aperture to illuminate the area such that the illuminated area emits fluorescent or luminescent light; and
  - a compound parabolic concentrator comprising a compound parabolic reflective surface and operative to collect and to direct light emitted from the illuminated area to an emission mirror.
51. The light transfer module of claim 50 wherein the emission mirror is operative to focus and to direct light from the compound parabolic concentrator to a monochromator as emission light.
52. The light transfer module of claim 50 wherein the emission mirror is a spherical mirror.
53. The light transfer module of claim 50 wherein the excitation mirror and the emission mirror are first-surface mirrors.
54. The light transfer module of claim 50 further comprising:
  - an optical filter operative to restrict the excitation light to a selected polarized plane; and
  - an optical filter holder selectively operative to insert the optical filter into the path of the excitation light.
55. The light transfer module of claim 54 further comprising:
  - a first polarizing filter operative to transmit light in a plane which is parallel to the plane of the polarized excitation light;

a second polarizing filter operative to transmit light in any plane which is not parallel to the plane of the polarized excitation light; and a polarizing filter holder selectively operative to interpose one of the first polarizing filter or the second polarizing filter between the compound parabolic concentrator and the emission mirror.

56. A method of analyzing a sample comprising:
- providing excitation light from a light source;
  - directing the excitation light through a first double monochromator;
  - transmitting the excitation light to the sample through a light transfer module;
  - employing the light transfer module to obtain light emitted by the sample;
  - directing the light emitted by the sample to a second double monochromator; and
  - analyzing light output by the second double monochromator.
57. The method of claim 56 wherein providing excitation light comprises implementing a spherical concave reflector system.
58. The method of claim 57 wherein providing excitation light comprises implementing an Offner 1:1 afocal relay.
59. The method of claim 56 wherein transmitting the excitation light to the sample comprises employing a first-surface excitation mirror positioned substantially coaxial with a sample holder supporting the sample.
60. The method of claim 56 wherein employing the light transfer module to obtain light emitted by the sample comprises utilizing a compound parabolic concentrator.
61. The method of claim 60 wherein directing the light emitted by the sample comprises focusing light from the compound parabolic concentrator to a first-surface emission mirror.
62. The method of claim 56 further comprising restricting the excitation light to a selected polarized plane.
63. The method of claim 62 further comprising selectively inserting one of a plurality of polarizing filters into the light path of the light emitted by the sample.
64. The method of claim 63 wherein the selectively inserting comprises interposing one of a plurality of polarizing filters between the sample and an emission mirror operative to direct the light emitted by the sample to the second double monochromator.



65. The method of claim 56 wherein the analyzing light output by the second double monochromator comprises detecting and analyzing more than one fluorescent compound in the sample.

Patent 6,366,001